CHANGES IN THE CONCENTRATION OF REDUCING SUBSTANCES DURING THE METAMORPHOSIS OF THE HOUSEFLY (MUSCA DOMESTICA LINNAEUS)*

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Frew (1929) studied changes in the glucose content during metamorphosis of the blowfly (species not given). His results indicate that glucose increases in concentration just prior to pupation, and decreases rapidly immediately afterward. He also reported the occurrence of sudden increases in glucose content for definite points during pupal life. These results have been interpreted as indicating that a synthesis of glucose occurs during metamorphosis. Frew suggests that the glucose is derived from protein during the first half and from fat the last half of the pupal period. Courtois-Drilhon (1931) reported an increase in the glucose content of the early pupae of three species of Lepidoptera (*Attacus pernyi, Sphinx ligustri* and *Saturnia pyri*). This increase continued from the time of pupation in August, until December, and was followed by a steady decrease until emergence the following April. Crescitelli and Taylor (1935) studied the bee-moth, *Galleria mellonella,* and found that the period of spinning (prepupal stage) is one during which increasing concentrations of reducing substances are found. During pupal life, which lasted for seven days at 30° C., the concentration of reducing substances decreases at first, then rises. A final drop in concentration occurs just prior to the emergence of the adult. Ludwig and Rothstein (1949) found that in the Japanese beetle, *Popillia japonica,* the glucose content decreased from 0.52 per cent in the 3rd instar larva to 0.42 per cent in the early prepupa, but increased in the newly-molted pupa to 0.79 per cent. During the first day of pupal life, at 25° C., there was a small but significant

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drop to 0.68 per cent. It remained constant for about 2 days and then increased slowly throughout the remainder of the pupal stage, reaching a value of 0.86 per cent in the 9-day pupa. Emergence was associated with an actual loss of glucose but, because of the loss of weight which occurs at that time, the percentage value increased. The increase in glucose and glycogen which was observed at the time of pupation was considered by the authors to be the result of a breakdown of insoluble proteins which occurs at that time, the degradation products being temporarily stored as carbohydrates.

This review indicates that the concentrations of reducing compounds may vary greatly in different insects during metamorphosis. In some forms there is an actual loss of this constituent; whereas in other forms its concentration increases significantly. Because of this variation, it was decided to study the changes in reducing compounds during the growth, metamorphosis and imaginal life of the housefly, *Musca domestica*.

MATERIAL AND METHODS

The larvae of the housefly were raised on whole milk. A finger-bowl containing cotton saturated with whole milk served as the feeding medium. Twenty-four hours after the eggs were laid on this saturated cotton, they were transferred to a humidifier regulated at approximately 30° C. and a humidity near saturation. The eggs hatched in approximately 24 hours under these conditions, and the time of hatching was recorded. In this manner carefully timed records, within 24 hours, were obtained for each group of experimental animals. Readings on glucose were made on groups of five larvae at the following stages: one, two, three, four, five and six days after hatching; on groups of four pupae, one, two, three and four days after puparium formation; and on groups of two newly-emerged adults and groups of two old-adults (7 to 10 days after emergence).

Determinations of reducing substances, expressed as glucose, were made by the Hagedorn and Jensen procedure (see Hawk, Oser and Summerson, 1947, p. 528).

OBSERVATIONS

The results of the glucose determinations are given in Table 1. The table contains the average weights of the insects and the aver-

age percentages (by weight) of glucose present at each day of growth and metamorphosis. These values are averages obtained from ten determinations. Since the amount of glucose is greater in the larger insects, the percentage values at different stages are considered more reliable than those for weights. There is no difference in the amount of glucose present in the one- and two-day larva. However, between the second and third days there is a decrease from 0.36 to 0.23 per cent. This decrease is followed by an increase in concentration in the four-day larva to 0.26 per cent.

TABLE 1

Stage in development	Number of insects	Average weight in mg. per group	Average weight of glucose in mg per group	Glucose as per cent weight and standard errors
1-day larvae	50	25.	9.0	0.36 ± .0085
2-day larvae	50	72.	25.7	0.36 ± .0289
3-day larvae	50	124.	28.0	0.23 ± .0066
4-day larvae	50	89.	23.4	0.26 ± .0106
5-day larvae	50	33.	5.5	0.17 ± .0069
6-day larvae	50	75.	20.6	0.27 ± .0117
1-day pupae	40	53.	19.5	0.37 ± .0142
2-day pupae	40	54.	21.0	0.39 ± .0157
3-day pupae	40	53.	21.3	0.40 ± .0174
4-day pupae	40	40.	16.9	0.42 ± .0177
newly-emerged adults	20	37.	31.2	0.84 ± .0108
old adults	20	17.	20.4	1.20 ± .0075

A second drop to 0.17 per cent occurs in the five-day larva, followed by an increase in the six-day larva to 0.27 per cent. The glucose then rises to its former value of 0.37 per cent following puparium formation. There is a steady increase in the concentration of glucose in the pupal stage reaching a high of 0.42 per cent in the four-day pupa. There is an increase in weight exhibited by the newly emerged adults, within the first 24 hours after emergence. This increase may be explained by the fact that these adults had already fed. However, as the adult ages, it loses weight resulting in a further increase in the percentage value of

glucose. Glucose increases in the adult stage from 0.84 in the newly-emerged to 1.20 per cent in the old adult.

In Table 2 is given a statistical analysis of these results. Beginning with the second day, there are significant changes between successive days of the larval period. In the pupal period, the

TABLE 2

Stages compared	Difference between means	Standard error of difference	Difference Standard error of difference
LARVAE			
1 and 3 day	0.13	±.0107	12.0
3 and 4 day	0.03	±.0125	2.4
4 and 5 day	0.09	±.0126	7.1
6 and 1 day pupa	0.10	±.0184	5.4
PUPAE			
1 and 2 day	0.02	±.0211	0.9
2 and 3 day	0.01	±.0234	0.4
3 and 4 day	0.02	±.0248	0.8
1 and 4 day	0.05	±.0231	2.1
ADULTS newly-emerged and old adults	0.36	±.0131	27.5

only statistical significance observed is that found when the one and four-day pupae are compared. A significant change is also found between the newly-emerged and old adults.

DISCUSSION

The irregular increases and decreases in the concentration of glucose throughout growth may have significance when correlated with the physiological activities of the organism. Thus, for example, an increase in the percentage of glucose from 0.37 per cent in the one-day pupa to 1.20 per cent in the old adult may be indicative of an endogenous synthesis of glucose, or other reducing compounds. Moreover, decreases in the concentration of glucose can similarly be associated with carbohydrate utilization.

The results indicate a gradual increase in the concentration of reducing compounds during the pupal period. Similar increases

were found by Frew (1929); Crescitelli and Taylor (1935) and Ludwig and Rothstein (1949). In the present study, there is no evidence of increases followed by decreases in concentration such as reported by Frew (1929); Courtois-Drilhon (1931); Crescitelli and Taylor (1935) and Ludwig and Rothstein (1949). Hitchcock and Haub (1941) in the blowfly *Phormia regina*, found a steady decrease in the concentration of reducing substances during the pupal period. These findings are in direct opposition with those found in the present study. However, there is agreement in the fact that the concentration of reducing substances increases with the transition from the pupa to the adult.

Frew (1929), correlated the marked synthesis of glucose during the pupal stage, with the respiratory quotient. If this glucose were all oxidized to CO_2 and H_2O, the expected respiratory quotient would be about 0.7 to 0.8 depending on whether the glucose is formed from protein or fat. Frew postulated that it is improbable that the glucose formed is entirely used in respiration; some must almost certainly be used in building up the growing imaginal tissue. Frew further stated that there is definite evidence of a vigorous synthesis of glucose during the whole of the pupal period. He holds that this must be regarded as the most probable explanation of the low respiratory quotient obtained, namely, 0.651. Ludwig (1931) showed a decrease in the rate of oxygen consumption during the last four or five days of the prepupal stage, which continued for several days of the pupal stage. The respiratory quotient of the larva varied from 0.7 to 0.97. It gradually decreased during metamorphosis and, in the pupa, varied from 0.4 to 0.7. He also stated that the low respiratory quotients may possibly be associated with the synthesis of glucose from protein or fat. Since the work of Ludwig and Rothstein (1949) indicates that glycogen supplies the energy required during the pupal stage, there seems to be no definite correlation between the respiratory quotient and the type of metabolism occurring in the insect. Taylor and Steinbach (1931) also give a discussion of the respiratory quotient and its importance during pupal metabolism. These authors point out that the oxidation of fats cannot occur without the oxidation of carbohydrate. Hence, it is false to assume that a quotient of 0.69 (the value obtained in their work) represents an exclusive oxidation of fat.

Schneiderman (1953) studying the respiration of diapausing pupa of the moth, *Platysamia cecropia*, found that metabolic carbon dioxide is retained within the insect and released during brief periods. He further states that the discontinuous release of carbon dioxide is apparently a widespread phenomenon in diapausing pupa. Schneiderman goes on to say that this fact is evidently responsible for the extremely low and apparently erroneous values reported for the respiratory quotients for diapausing pupa (0.78 found by Schneiderman, over a two day period). Buck, Keister and Specht (1953) confirm these results using pupae of *Agapema* (species not given), during diapause.

To the author's knowledge no other studies have been made on the concentration of reducing compounds during the larval growth of insects.

SUMMARY

Determinations were made on the glucose content of the housefly *Musca domestica*, at the following stages: one, two, three, four, five and six-day larvae; one, two, three and four-day pupae; newly-emerged and old adults.

The glucose content of the one-day larva was 0.36 per cent. It decreased to 0.23 per cent in the three-day larva, and to 0.17 in the five-day larva. It then increased to 0.37 per cent in the one-day pupa. The per cent of glucose increased during the pupal stage, reaching 0.42 per cent in the four-day pupa. At the time of emergence there is a loss of weight. However, the percentage value of glucose is increased from 0.84 in the newly-merged adults to 1.20 per cent in the late adults.

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CHANGE IN PUBLICATION ARRANGEMENTS

Beginning with Volume 62 of the Journal, the Society found it necessary to change publishers. Announcement of and reasons for the change were given in Vol. 62, (2) June, 1954, p. 98.

Unfortunately, it soon became apparent that equal service to that provided by the former publishers could not be achieved. Unreasonable delays occurred, in spite of every effort on the parts of the editor and the various officers of the Society to maintain a reasonable publication schedule. The four issues of volume 62 were published over a period of more than two years. Obviously this was not fair to the subscribers nor to the Society and it became necessary finally, to again make a change in publishers.

The situation has been met by return of the Journal to the previous publishers, Business Press Inc. The entire Volume 63 is here presented in one issue. It is planned also to publish Volume 64 (1956) in one issue and, if possible, to revert back to four separate issues with the 1957 Volume, 65.

Throughout this difficult period your officers have been very much encouraged with the show of patience and understanding by the subscribers. It is the desire of the editor, other officers and Business Press to present the Journal on time, just as soon as possible.

The Volume Title Page, Table of Contents and Index to the Volume were omitted from the December 1954 issue. Publication in that issue would have resulted in further delay. This volume carries Title Pages, Tables of Contents and Indices for both Volumes 62 and 63. It is hoped that this might prove satisfactory for those subscribers who desire to bind the Journal. —F. A. Soraci.